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Analytical Methodology for the Determination of Chlorophenols in Human and Environmental Samples

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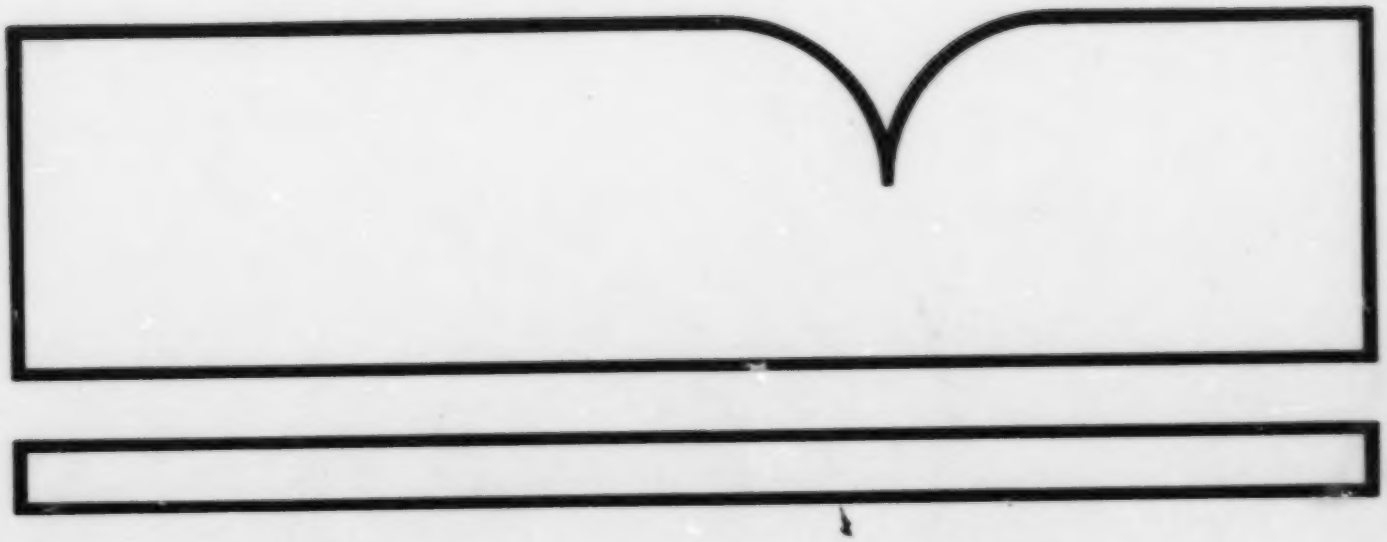

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Users Guide

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Analytical Chemistry Branch
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Research Triangle Park, NC 27711

August 27, 1980

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This document deals with the methodology for the determination of chlorophenols in human and environmental media through the use of blood, urine, and air samples.

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Methodology is presented for the determination of chlorophenols in human and environmental media. The methodology for air samples is a modification of the analysis of chlorophenols in blood and has not been fully investigated as to quantitative reliability.

Instrumentation Required

The liquid chromatographic system consists of a Tracor model 995 isochromatographic pump with a model CV-1-UHPa-HC Valco valve and a model LC-2A electrochemical detector from Bioanalytical Systems. The electrochemical detector is operated at an oxidation potential of +1.1 V. The electrode used is either carbon paste or glassy carbon. Zorbax® C8 columns of 15 or 25 cm length are used. Two different mobile phases must be used to determine all chlorophenol congeners since gradient elution cannot be used with the electrochemical detector.

The mobile phase used for the early eluting phenols is 46% 0.05 M, pH 4.0 phosphate buffer, 40% methanol, 14% acetonitrile. The mobile phase used for the later eluting phenols is 50% phosphate buffer, 50% acetonitrile. All solvents are chromatographic grade and are filtered through 0.4 micron filters before use. All necessary phenol standards can be obtained from Aldrich Chemical Co.

Analysis of Urine Samples

The samples are prepared for analysis by the method of Edgerton et al.[1] Briefly, a 4 mL aliquot of urine is placed in a 15-mm x 125-mm screw cap culture tube, 1 mL of concentrated hydrochloric acid is added, and the tube is heated in a boiling water bath for one hour. The

hydrolyzed urine is tranferred to a XAD-4 macroreticular resin column. After several aqueous washes, the chlorinated phenols are eluted with 10% 2-propanol in hexane. This eluant is concentrated to appropriate volumes and analyzed by EC-GC and by electrochemically detected HPLC. The organic solvent extract containing the chlorinated phenols from the above method is partitioned with 0.1 N NaOH. The volume of 0.1 N NaOH is equal to the volume of the original urine sample. The aqueous layer containing the phenols is transferred to a 13 ml graduated centrifuge tube and acidified with concentrated H_3PO_4 to a pH of 3 to 4 as determined with pHydrion paper. An aliquot from the aqueous phase is then injected into the liquid chromatograph.

Analysis of Blood Samples

The method reported by Lores, et al.[2] for the determination of halogenated anilines in urine by high-performance liquid chromatography with an electrochemical detector (HPLC/ECD) is modified and used for analysis of chlorophenol residues in blood. Whole blood samples (3-7 g) are hydrolyzed in dilute hydrochloric acid solution before the phenolic compounds are extracted into 1:1 (v/v) hexane/ethyl ether. The organic extracts are then partitioned with sodium hydroxide solution and the aqueous alkaline solutions containing the phenols are buffered by addition of phosphoric acid (amount dependent upon concentration of chlorophenols anticipated) before portions are analyzed by HPLC/ECD under isocratic elution conditions.

The response and instrumental noise level of the electrochemical detector varied slightly from day to day so that a fixed limit of measurement cannot be quoted for the method. Instead, the limit of detection

for the instrument is defined as the response of each phenol analyzed required to produce a signal to noise ratio of five; usually it is at 5 ng injected.

Phenol and 2-chlorophenol (2-CP) cannot be analyzed using this method. Fortification-recovery experiments are shown in Table 1.

Analysis of Air Samples

The method for the determination of chlorophenols in blood by HPLC/ECD is modified and used for analysis of chlorophenol residues in air. Polyether-type polyurethane foam is used to collect air samples according to Environmental Protection Agency "Protocol for Assessment of Human Exposure to Airborne Pesticides.[3] The methods for chlorophenols involve the following steps:

1. Remove the foam plug with forceps and place into Soxhlet extractors.
2. Extract with an appropriate volume of 5% diethyl ether (stabilized with 2% ethanol, Mallinkrodt 0850) in hexane for 50-100 cycles in the extractor.
3. Remove the boiling flask to a rotary evaporator and reduce the solvent volume to approximately 5 mL. (CAUTION: Do not let sample go dry.)
4. Transfer the concentrate to a graduated centrifuge tube with rinsing and concentrate volume to exactly 2mL.
5. Partition the extract into appropriate volumes of 0.1 N aqueous sodium hydroxide, which when buffered with phosphoric acid will permit maximum detectability of chlorophenols.
6. Analyze buffered solution according to the procedure described above.

Table 1. Recoveries of Chlorophenal Standards Added to Whole Blood[a]

Sample		3-CP	4-CP	2,4-DCP	2,4,6-TCP	2,4,5-TCP	PCP
ACB pooled blood		-[b]	-	-	-	-	3[c]
ACB pooled blood, low level fortification amounts							
found	1	36.2	44.2	47.3	36.2	52.6	80.0
	2	38.4	41.7	38.4	39.3	55.7	97.8
	3	42.3	48.3	46.5	34.5	46.9	56.6
	4	42.2	47.1	44.4	36.4	44.4	76.8
	5	43.7	36.7	46.2	38.2	52.2	115
Average amount found		40.6	43.6	44.6	36.9	50.4	85.3
Amount added		50.7	44.8	47.2	45.2	50.4	105
Average percent recovery		80	97	94	82	100	81
RSD[d]		6.2	11	8.1	5.1	9.1	26
ACB pooled blood, high level fortification amounts							
found	1	450	380	425	346	400	1008
	2	460	380	450	345	460	760
	3	649	428	428	391	438	695
	4	447	408	384	384	413	549
	5	440	419	393	419	450	1013
Average amount found		489	403	416	377	432	805
Amount added		507	448	472	452	504	1050
Average percent recovery		96	90	88	83	86	77
RSD		18	5.5	6.5	8.4	5.8	25
Overall average percent recovery (N=10)		88	94	91	83	93	79
RSD		17	9.2	7.9	6.7	11	24

[a]Fortified blood samples weighed from 4.5 to 5.5 g and the amounts of chlorophenols added and found are given in ng.
[b]A dash indicates not present at the limit of detection for the method which was set at a signal to noise ratio of 4.
[c]This value was used to correct the amounts found.
[d]RSD = relative standard deviation (coefficient of variation).

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